induced MPT, respiration, and phospholipid fatty acyl composition. Both DHA and EPA enriched diets lowered circulating free fatty acids and triglycerides by approximately 40% ($p<0.05$, DHA vs CTRL and EPA vs CTRL, NS, DHA vs EPA). DHA supplementation increased DHA by 63% ($p<0.05$ vs control) and decreased ARA by 61% ($p<0.05$ vs control) in mitochondrial phospholipids, and significantly delayed MPTP opening (57% more calcium necessary to induce MPTP vs CTRL, $p<0.05$). EPA supplementation did not affect DHA, only modestly lowered ARA (−33% vs CTRL, $p<0.05$), and had no effect of MPTP opening. State 3 respiration with a variety of substrates was unaffected by dietary treatment, however DHA decreased state 4 respiration by 30% and the increased RCR by 70% with pyruvate + malate as the substrate, both in the absence and presence of oligomycin ($p<0.05$); treatment with EPA had no effect. The P:O ratio was not different among groups with any of the substrates. In summary, DHA supplementation favorably altered mitochondrial phospholipid composition and delayed MPT in cardiac mitochondria, while EPA had no effect. These effects may contribute to the protection against heart disease with ω-3 PUFA supplementation, and suggest that supplementation with DHA should be superior to EPA.

doi:10.1016/j.bbabio.2010.04.243

9P.6 Nitrolinoleate modifies ANT, K_{ATP} channels and complex II and modulates their activity

Sergiy M. Nadtochiy[1], Andrew P. Wojtovich[2], Paul S. Brookes[1]
[1]*University of Rochester, Department of Anesthesiology, USA*
[2]*University of Rochester, Department of Pharmacology and Physiology, USA*
E-mail: Sergiy_Nadtochiy@urmc.rochester.edu

Nitroalkenes are electrophilic molecules which can cause post-translational modifications of proteins and modulate their functional activity. Previously we demonstrated endogenous formation of nitrated linoleate (LNO_2) in mitochondria isolated from perfused heart after ischemic preconditioning. In addition, synthetic LNO_2 protected isolated cardiomyocytes against simulated ischemia/reperfusion injury. Biotin-tagged LNO_2 replicated this cardioprotective effect, and caused reversible modification of mitochondrial proteins. Thus, we hypothesized that mitochondrial targets of LNO_2 might play an important role in cardioprotection. Previously we demonstrated that LNO_2 induced mitochondrial H^+ leak *via* modification of ANT. Further studies revealed that LNO_2 (1 μM) opened mitochondrial K_{ATP} channels in a 5-HD and glybenclamide sensitive manner. Although the molecular identity of the mK_{ATP} channel has not been fully elucidated, we previously showed that complex (Cx) II might be involved in regulation of mK_{ATP} channel activity. We found that LNO_2 physically interacted with the 70 kDa subunit of Cx II and inhibited its enzymatic activity. Notably, the cardioprotective effects of mild H^+ leak, opening of mK_{ATP} channels and reversible inhibition of the respiratory chain are well documented. Thus, our findings characterize LNO_2 as a pleotropic molecule which might recruit several protective mitochondrial pathways to elicit cardioprotection.

doi:10.1016/j.bbabio.2010.04.244

9P.7 Doxorubicin-induced cardiac, hepatic and renal mitochondrial toxicity in an acute *versus* sub-chronic treatment model

Gonçalo C. Pereira[1], Susana P. Pereira[1], Claudia V. Pereira[1], José A. Lumini[2,3], José Magalhães[2], António Ascensão[2], Maria S. Santos[1], James A. Bjork[4], António J. Moreno[5], Kendall B. Wallace[4], Paulo J. Oliveira[1]
[1]*Center for Neurosciences and Cell Biology, Department of Life Sciences, University of Coimbra, Portugal*
[2]*Research Centre in Physical Activity, Health and Leisure, Faculty of Sport Sciences, University of Porto, Porto, Portugal*
[3]*Faculty of Health Sciences, University of Fernando Pessoa, Porto, Portugal*
[4]*Department of Biochemistry and Molecular Biology, University of Minnesota Medical School, Duluth, USA*
[5]*Institute for Marine Research, Department of Life Sciences, University of Coimbra, Coimbra, Portugal*
E-mail: goncalopereira@ci.uc.pt

Nowadays, Doxorubicin (DOX) is probably one of the most effective anticancer drugs available in the clinic. However, the treatment is usually followed by a cumulative and persistent cardiotoxicity. Mitochondria have a critical role in DOX-mediated toxicity however there are still doubts whereas mitochondrial toxicity is specific to the heart. Therefore, the present work characterizes two different models of toxicity (acute vs. sub-chronic), regarding mitochondrial physiology from three different tissues (heart, liver and kidney) from treated rats. Wistar rats were sub-chronically (7 wks, 2 mg/kg) or acutely (20 mg/kg) treated with DOX and allowed to rest one week or 24 h after the last injection, respectively. Sub-chronically-treated animals showed a decrease in body mass gain during treatment while no changes were observed in acute model. Plasma profile from both models was altered but the sub-chronic treatment presented the most dramatic changes. Histological analysis revealed the presence of lipid droplets in liver slices from acutely treated rats. Regarding mitochondrial bioenergetics, differences between saline and DOX-treated rats were observed: in the acute model, differences included state 3 respiration in the liver and kidney and the ADP/O in the heart. In the sub-chronic model, differences regarding state 3 respiration in the heart and kidney was observed. We also determined that cardiac mitochondria from sub-chronic-treated animals presented a lower calcium loading capacity, which was not observed in the other tissues. However, gene expression analyses showed no alterations in the chronic model but interestingly, decreased mRNA levels for the ANT, VDAC and increased CyP-D mRNA were detected in the acute model. Aconitase activity, a sensitive marker of oxidative stress, was decreased in the kidney (acute model) and in the heart (sub-chronic model). In conclusion, data confirm that mitochondrial alterations result from DOX treatment, being more severe in the heart and are very dependent on the treatment protocol. It remains to be determined if mitochondrial alterations in organs such as liver and kidneys are a specific and direct effect of DOX on mitochondria or if they result of secondary effects of DOX on other targets.

The present work is supported by the Portuguese FCT (SFRH/BD/36938/2007 to GP, PTDC/SAU-OSM/64084/2006 and PTDC/SAU-OSM/104731/2008 to PO).

doi:10.1016/j.bbabio.2010.04.245

9P.8 Glycine regulates calcium capacity of isolated brain mitochondria

Alexey A. Selin[1], Natalia V. Lobysheva[1,2], Yaroslav R. Nartsissov[1], Lev S. Yaguzhinsky[2]
[1]*Institute of Cytochemistry and Molecular Pharmacology, Russia*
[2]*Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Russia*
E-mail: alexey.a.selin@gmail.com

Glycine, inhibitory neurotransmitter, has been found to be effective against neuronal cell death in *in vivo* and *in vitro* models of ischemic stroke. We have shown that glycine prevented respiratory index depletion of mitochondria in the homogenate of the cerebral cortex after 24 h common carotid artery occlusion in rats, along with preventing the

activation of caspase 3. Glycine also maintained phosphorylating ability of mitochondria after incubation of rats' brain cortex slices under anoxia for 30 min. Neuronal death during ischemic stroke mediated by glutamate excitotoxicity which results in elevation of intracellular calcium concentration. Elevated concentrations of calcium induce mitochondrial permeability transition pore, which dissipates mitochondrial electrochemical gradient and lead to energy collapse. Therefore we investigated the effect of glycine to influence directly on calcium capacity of isolated mitochondria in conditions close to brain tissue surviving during ischemic stroke. We studied the calcium capacity of isolated brain mitochondria after incubation under anoxia at different temperatures and the effect of glycine on this parameter. Concentration of calcium in the incubation medium and the mitochondrial membrane potential were measured. Incubation of the mitochondria at room temperature (22 °C) under 30 min of anoxia led to a decrease of the calcium capacity of mitochondria by 80–90% compared with intact mitochondria, also significantly decreased sensitivity to cyclosporin A. Calcium capacity at the same conditions and in the presence of glycine 5 mM was reduced only by 50–60%. There was a concentration dependence of this effect and it could be observed under not less than 2 mM glycine. Our data show that glycine prevents decrease of calcium capacity in isolated brain mitochondria during anoxic conditions. These findings suggest a novel mechanism for glycine as a potential stroke therapeutic.

doi:10.1016/j.bbabio.2010.04.246

9P.9 Evaluation of neuroprotective abilities of the novel mitochondria-targeted antioxidants

Mariya I. Danshina[1], Denis N. Silachev[1], Irina B. Pevzner[1], Egor Y. Plotnikov[1], Yuri B. Pirogov[2], Nikolaj K. Isaev[1], Vladimir P. Skulachev[1], Dmitry B. Zorov[1]
[1]*A.N.Belozersky Institute of Physico-Chemical Biology and Institute of Mitoengineering, Moscow State University, Russia*
[2]*Training and Research Interfaculty Center of Magnetic Tomography and Spectroscopy, Moscow State University, Russia*
E-mail: proteins@mail.ru

Over the world, brain ischemia is one of the most common causes of death and adult disability. Oxidative stress is known to be highly associated with brain ischemia with an important role of mitochondria as a major source of reactive oxygen species. Therefore, therapeutic approaches targeting mitochondrial dysfunction and oxidative damage hold great promise in neurodegenerative diseases. We tested mitochondria-targeted chimeric compounds carrying antioxidant moiety as potential agents to efficiently alleviate the deleterious consequences of ischemic insult. Among all tested compounds the highest efficiency was displayed by SkQR1 consisting of a rhodamine moiety linked to a plastoquinone residue. Brain ischemia in rats was induced by insertion of a silicon-coated thread in the middle cerebral artery (MCA). The volume of brain infarct was determined on the first postoperative day by magnetic resonance imaging. Behavioral test was performed 1 day before the surgery and on the first day after the induction of ischemia. Measuring the proteins content in the brain homogenate tissue was determined by Western blotting. We found that a single intraperitoneal injection of SkQR1 at the concentration of 0.5, 1, 2 mM/kg before and after MCA occlusion significantly diminishes infarct volume and improves performance of a test characterizing neurological deficit of ischemic animals in a dose-dependent manner. An analog of SkQR1 without plastoquinone did not display apparent neuroprotective properties. We also revealed that SkQR1 activates signaling pathways involved in ischemic tolerance induction. We conclude that beneficial effect of rhodamine derivative of mitochondria-targeted compound SkQR1 causing significant improvement of neurological functions and decreased infarct volume may be explained by a direct antioxidative effect of the drug. However, we cannot exclude some other mechanisms of SkQR1 action, in particular, through a mechanism of ischemic tolerance induction.

doi:10.1016/j.bbabio.2010.04.247

9P.10 Oxidative inactivation of mitochondrial creatine kinase: Differential sensitivity of isoforms

Malgorzata Tokarska-Schlattner, Uwe Schlattner
Laboratory of Fundamental and Applied Bioenergetics (LBFA), Inserm U884, University Joseph Fourier - Grenoble 1, Grenoble, France
E-mail: malgorzata.tokarska@ujfrenoble.fr

Isoforms of creatine kinase (CK) are key players in energy metabolism of many cells with high and/or fluctuating energy demands by providing energy buffer and energy transfer functions. They are easily inactivated in situations of oxidative stress, which makes them a critical factor for energy failure occurring in many related pathologies. Reactive oxygen and nitrogen species (ROS, RNS) not only induce enzymatic inactivation, which occurs with all CK isoenzymes, but also specific damage to the mitochondrial CK isoforms (MtCKs). This includes impairment of critical MtCK properties like destabilization of the native octameric state or decreased membrane binding capacity [1]. Using purified recombinant proteins, cell homogenates and mitochondria isolated from rat heart and brain, we have compared sarcomeric sMtCK (expressed in heart and skeletal muscle) and ubiquitous uMtCK (expressed in many other tissues) with respect to their sensitivity to oxidative inactivation induced by the drug doxorubicin or occurring spontaneously after extraction under non-reducing condition. Sarcomeric sMtCK showed significantly higher sensitivity to oxidation and was the isoform responsible for the loss of CK activity in heart extracts upon storage under non-reducing conditions. The sMtCK dimer was more easily inactivated as compared to the octamer, and solubilization of sMtCK from membrane (promoting dimerization) made the protein an especially vulnerable substrate for inactivation. This differential susceptibility of the two MtCK isoenzymes has been related to some differences in their molecular structures (e.g. number and surface exposure of cysteine residues). It may contribute to energy deficits that occur in oxidatively stressed heart expressing the sMtCK isoform [2,3].

References

[1] Schlattner U, Tokarska-Schlattner M, Wallimann T (2006) *Biochem. Biophys. Acta* **1762:** 164-180.
[2] Tokarska-Schlattner M, Zaugg M, Zuppinger C, Wallimann T, Schlattner U (2006) *J. Mol. Cell. Cardiol.* **41:** 389-405.
[3] Tokarska-Schlattner M, Dolder M, Gerber I, Speer O, Wallimann T, Schlattner U (2007) *Biochim. Biophys. Acta* **1767:** 1276-1284.

doi:10.1016/j.bbabio.2010.04.248

9P.11 Liver mitochondria and insulin resistance

Guillaume Vial[1], Hervé Dubouchaud[1], Karine Couturier[1], Cécile Cottet-Rousselle[1], Nellie Taleux[1], Anne Athias[2], Anne Galinier[3], Louis Casteilla[3], Xavier M. Leverve[1]
[1]*Inserm, U884, Grenoble, F-38041 France, Université J. Fourier Grenoble-1, Bioénergétique Fondamentale et Appliquée, Grenoble, F-38041 France*
[2]*Inserm, UMR866, Dijon, F-21079 France, Université de Bourgogne, Faculté de Médecine, Institut Fédératif de Recherche Santé-STIC, Dijon, F-21079 France*
[3]*CNRS, UMR5241, Toulouse, F-31432 France; Université Paul Sabatier Toulouse, Toulouse, F-31432 France*
E-mail: Guillaume.vial@ujfrenolble.fr